COMPOSITIONS CONTAINING SOLUBLE FORMS OF HLA-G IN THE TREATMENT OF INFLAMMATORY SKIN PATHOLOGIES

The present invention relates to the use of compositions containing soluble forms of HLA-G in the treatment of skin pathologies, and in particular of inflammatory dermatoses, to the method for obtaining said soluble forms of HLA-G and also to the antibodies directed against said soluble forms.

The antigens of the major histocompatibility complex (MHC) are divided up into several classes, the class I antigens (HLA-A, HLA-B and HLA-C) which have 3 globular domains (α1, α2 and α3), the α3 domain of which is associated with β2 microglobulin, the class II antigens (HLA-DP, HLA-DQ and HLA-DR) and the class III antigens (complement).

The class I antigens comprise, besides the abovementioned antigens, other antigens, termed unconventional class I antigens, and in particular the HLA-E, HLA-F and HLA-G antigens; the latter, in particular is expressed by extravillous trophoblasts of normal human placenta and thymic epithelial cells.

The sequence of the HLA-G gene (HLA-6.0 gene) has been described by GERAGHTY et al., (Proc. Natl. Acad. Sci. USA, 1987, 84, 9145-9149); it comprises 4396 base pairs and has an intron/exon organization homologous to that of the HLA-A, -B and -C genes. More precisely, this gene comprises 8 exons, 7 introns and a 3' untranslated end; the 8 exons correspond, respectively, to: exon 1: signal sequence, exon 2; α1 extracellular domain, exon 3: α2 extracellular domain, exon 4: α3 extracellular domain, exon 5: transmembrane region, exon 6: cytoplasmic domain I, exon 7: cytoplasmic domain II (untranslated), exon 8: cytoplasmic domain III (untranslated) and 3' untranslated region (GERAGHTY et

al., mentioned above; ELLIS et al., J. Immunol., 1990, 144, 731-735; KIRSZENBAUM M. et al., *Oncogeny of hematopoiesis, Aplastic anemia* Eds. E. Gluckman, L. Coulombel, Colloque INSERM/John Libbey Eurotext Ltd). However, the HLA-G gene differs from the other class I genes in that the in-frame translation termination codon is located at the second codon of exon 6; consequently the cytoplasmic region of the protein encoded by this HLA-6.0 gene is considerably shorter than that of the cytoplasmic regions of the HLA-A, -B and -C proteins.

These HLA-G antigens are essentially expressed by the cytotrophoblastic cells of the placenta and are considered to play a role in protecting the fetus (lack of rejection by the mother). In addition, in so far as the HLA-G antigen is monomorphic, it may also be involved in the growth or the function of placental cells (KOVATS et al., Science, 1990, 248, 220-223).

Other investigations concerning this unconventional class I antigen (ISHITANI et al., *Proc. Natl. Acad. Sci.* USA, 1992, 89, 3947-3951) have shown that the primary transcript of the HLA-G gene may be spliced in several ways and produces at least 3 distinct mature MRNAs: the primary HLA-G transcript provides a complete 1200 bp copy (G1), a 900 bp fragment (G2) and a 600 bp fragment (G3).

The G1 transcript does not comprise exon 7 and corresponds to the sequence described by ELLIS et al. (mentioned above), i.e. it encodes a protein which comprises a leader sequence, three external domains, a transmembrane region and a cytoplasmic sequence. G2 mRNA does not comprise exon 3, i.e. it encodes a protein in which the α1 and α3 domains are directly joined; the G3 mRNA does not contain exon 3 or exon 4, i.e. it encodes a protein in which the α1 domain and the transmembrane sequence are directly joined.

The splicing which prevails in order to produce the HLA-G2 antigen leads to the joining of an adenine (A) (originating from the domain encoding α1) with an AC sequence (derived from the domain encoding α3), which leads to the creation of an AAC codon (asparagine) in place of the GAC codon (aspartic acid) encountered at the beginning of the sequence encoding the α3 domain in HLA-G1.

The splicing generated in order to produce HLA-G3 does not lead to the formation of a new codon in the splicing zone.

The authors of that article also analyze the various proteins expressed: the 3 mRNAs are translated into protein in the .221-G cell line.

Some of the inventors have shown the existence of other spliced forms of HLA-G mRNA: the HLA-G4 transcript, which does not include exon 4; the HLA-G5 transcript, which includes intron 4, between exons 4 and 5, thus causing a modification of the reading frame when this transcript is translated and, in particular, the appearance of a stop codon after amino acid 21 of intron 4; and the HLA-G6 transcript, which contains intron 4 but which has lost exon 3 (KIRSZENBAUM M. et al., *Proc. Natl. Acad. Sci.* USA, 1994, 91, 4209-4213; European Application EP 0 677 582; KIRSZENBAUM M. et al., *Human Immunol.*, 1995, 43, 237-241; MOREAU P. et al., *Human Immunol.* 1995, 43, 231-236). They have also shown that these various transcripts are expressed in several human fetal and adult cell types, in particular in lymphocytes (KIRSZENBAUM M. et al., *Human Immunol.*, 1995, mentioned above; MOREAU P. et al., *Human Immunol.* 1995, mentioned above).

There are therefore at least 6 different HLA-G mRNAs which potentially encode 6 protein isoforms of HLA-G, 4

of which are membrane-bound (HLA-G1, G2, G3 and G4) and 2 of which are soluble (G5, G6).

It has been shown that the expression of said protein isoforms of HLA-G is increased by γ-interferon (Yang et al., J. Immunol., 1996, **156**, 4224-4231).

The immunomodulatory function exercised by these HLA-G molecules has been described and the mechanisms of this function have been elucidated by demonstrating their interaction with lysis-inhibiting receptors present on NK cells (KIR receptors), leading to inhibition of the cytotoxic functions of these cells; for example, N. ROUAS-FREISS ET AL. (Proc. Nat. Acad. Sci., 1997, **94**, 5249-5254) have shown that K562 (human erythroleukemia line) target cells transfected with the HLA-G gene are protected against lysis by NK cells. These cells are normally sensitive to NK cells.

Some of the inventors have shown that NK cells do not express any HLA-G transcript (Teyssier et al., Nat. Immunol., 1995, **14**, 262-270), this result confirming that the products of expression of the HLA-G gene probably play a role in states of physiological immunotolerance (pregnancy) or pathological immunotolerance in which NK cells are particularly active (autoimmune diseases, transplants) or, on the contrary, are inhibited (abnormal presence of HLA-G on certain tumors or during viral infections).

Thus, some of the inventors have also shown that certain tumors express HLA-G molecules, which enables them to evade immune surveillance (Paul et al., Proc. Nat. Acad. Sci., 1998, **95**, 4510-4515).

Psoriasis is a common chronic inflammatory pathology, and is observed in 2% of individuals in Caucasian populations. Although the disease is characterized by hyperproliferation of the keratinocytes of the

epidermis, a large number of physiopathological studies have made it possible to show that T lymphocytes of the Th1 subtype which infiltrate the site of the lesions and produce interleukin 2 (IL-2) and γ-interferon (γ IFN), play a predominant role in the pathogenesis of this disease (Z. Bata-Csorgo et al., J. Investigative Dermatol., 1995, 89S-94S; Schlaak et al., J. Invest. Dermatol., 1994, **102**, 145-149; Vogel et al., Eur. J. Biochem., 1995, **227**, 143-149). It has been shown that the supernatant of T-cell clones derived from psoriasis lesions is capable of inducing hyperproliferation of the epidermis (Strange et al., J. Invest. Dermatol., 1993, **101**, 695-700). Similarly, it has been shown that maintenance of the psoriatic phenotype of human skin biopsies transplanted into SCID mice is dependent on the coinjection of T lymphocytes derived from said lesions (Gilbar et al., J. Invest. Dermatol. 1997, **109**, 283-288).

The expression of HLA-G in skin cells and its potential roll in the associated pathologies has been studied by Ulbrecht et al. (Eur. J. Immunol., 1994, **24**, 176-180). The authors of that article, who analyze only the expression of the transcripts of the membrane-bound isoforms of HLA-G, and in particular of the dominant isoform HLA-G1, show that, while a low level of HLA-G transcripts is detected in skin biopsies exhibiting psoriasis lesions, HLA-G transcripts are either absent or present at a high level in healthy skin biopsies derived from different individuals. These authors therefore conclude that there is no obvious link between the expression of HLA-G and skin pathologies.

The inventors have now found that soluble forms of HLA-G have a therapeutic action on inflammatory pathological skin conditions.

A subject of the present invention is the use of a composition essentially consisting of at least one

soluble form of HLA-G and of at least one pharmaceutically acceptable vehicle (excipient), for preparing a medicinal product for treating inflammatory pathological skin conditions.

The excipients combined with said composition are suitable for the desired route of administration; they are selected from excipients known to those skilled in the art.

Said soluble HLA-Gs may advantageously be administered generally (orally, parenterally) or locally (topical administration).

In the latter case, said composition is in the form of cream, of lotion, of liposomes or of gel.

The inventors have now found, unexpectedly, the presence, in inflammatory skin lesions, both of macrophages expressing the HLA-G protein, which are located in the dermal papillae, and of infiltrating $CD3^+$ T lymphocytes expressing a receptor for inhibiting cytotoxic functions which is recognized by HLA-G, such as for example the ILT2 receptor.

The inventors have shown that the dominant membrane-bound isoform HLA-G1 and the soluble isoform HLA-G5 are expressed only in inflammatory skin lesions, whereas no HLA-G protein is detected in healthy skin.

The inventors have also shown that the HLA-G protein, and in particular an isoform comprising at least the $\alpha 1$ domain of HLA-G, is capable of inhibiting the proliferative functions and the cytotoxic functions of T lymphocytes.

Thus, the inventors have therefore demonstrated the anti-inflammatory role of the HLA-G protein, and in particular of a composition consisting of a diffusible

soluble form comprising at least the α1 domain of HLA-G, such as for example the HLA-G5 isoform, in the treatment of inflammatory skin pathologies.

In accordance with the invention, said composition is particularly suitable for treating psoriasis.

According to an advantageous embodiment of the invention, said soluble form of HLA-G is selected from the group consisting of the soluble isoforms of HLA-G comprising at least one extracellular domain (α1, α2 or α3) and the solubilized forms of HLA-G1, HLA-G2, HLA-G3 or HLA-G4 (membrane-bound isoforms).

Said soluble HLA-Gs comprise at least the α1 extracellular domain.

Said soluble forms are in particular produced in a *baculovirus*.

With regard to the membrane-bound forms, they are advantageously expressed in eukaryotic cells, in accordance with the method described in International Application PCT WO 98/37098, and then solubilized by treatment of the membrane (stripping agent, such as papain) and suitable purification, for example on an immunoaffinity column with specific antibodies.

The term "soluble form of HLA-G" is intended to mean both the soluble HLA-Gs (not comprising a transmembrane domain) and the membrane-bound HLA-Gs which have been solubilized, for example under the conditions specified above.

Preferably, said composition, administered topically, comprises between 0.1 and 5 µg/ml, preferably between 0.5 and 2.5 µg/ml, of soluble form of HLA-G.

A subject of the present invention is also a method for preparing a soluble HLA-G, characterized in that it comprises the following steps:

- coinfecting insect cells with a baculovirus containing the β_2M cDNA and another baculovirus containing the α chain of a soluble isoform of HLA-G;

- culturing the transfected insect cells, and

- harvesting the supernatants and purifying the soluble isoform of HLA-G expressed.

According to an advantageous embodiment of said method, said soluble isoform of HLA-G is purified using an antibody specific for the soluble isoforms of HLA-G.

According to an advantageous arrangement of this embodiment, said antibody is obtained by immunizing nonhuman mammals, such as rabbits, with an immunogenic peptide consisting of a 21 amino acid synthetic peptide corresponding to the C-terminal portion encoded by intron 4 of the soluble HLA-G forms, the sequence of which is SKEGDGGIMSVRESRSLSEDL (SEQ ID NO:1), coupled to the KLH carrier protein.

Besides the above arrangements, the invention also comprises other arrangements, which will emerge from the following description which refers to examples of implementation of the method which is the subject of the present invention and also to the attached drawings, in which:

- figures 1 and 2 illustrate the presence of the RNAs of the various isoforms of HLA-G in psoriasis lesions, compared with healthy skin.

- figure 3 illustrates the presence of the RNA specific for the HLA-G5 isoform in psoriasis lesions, compared with healthy skin.

- figure 4 illustrates the inhibitory activity of the HLA-G isoforms on *natural killer* cells (NK cells) present in peripheral blood; this figure comprises, on the x-axis, the isoform studied and, on the y-axis, the percentage specific lysis. M8 cells (HLA class I^+, class II^- melanoma line cells) transfected either with the vector alone (M8-pCDNA) (Invitrogen) or with the vectors containing the cDNA encoding HLA-G1 (M8-HLA-G1), the cDNA encoding HLA-G2 (M8-HLA-G2), the cDNA encoding HLA-G3 (M8-HLA-G3) or the cDNA encoding HLA-G4 (M8-HLA-G4) are used as targets (T). Peripheral blood monoclear cells, PBMCs, are used as effector cells (E). The results are expressed as percentage lysis, recorded in 4 h in a chromium 51(^{51}Cr)-release assay.

- figure 5 illustrates the inhibitory activity of the HLA-G isoforms on a CD8+ T lymphocyte line restricted for HLA-A2 presenting a viral peptide originating from the matrix of the influenza virus, positions 58 to 66; this figure comprises, on the x-axis, the isoform studied and on the y-axis, the percentage specific lysis; the effector cell/target cell ratio is 15:1.

It should be clearly understood, however, that these examples are given only by way of illustration of the subject of the invention, for which they in no way constitute a limitation.

EXAMPLE 1 : Production of a soluble form of an isoform of HLA-G in a baculovirus

The soluble HLA-G5 isoform, the structure of which consists of 3 extracellular domains, α1, α2 and α3, associated with β2-microglobulin (β2M), is derived from an alternative transcript which has a stop codon in

intron 4 of the HLA-G gene. The production of a recombinant soluble HLA-G protein makes use of coinfection of the insect cells with a baculovirus containing the complementary DNA of β2M, and another baculovirus containing the cDNA of the α chain of HLA-G5. This in fact makes it possible to obtain the physiological form HLA-G5/β2M.

1. Construction of transfer vectors for recombination with the BacTen virus.

a- Insertion of the β2M gene into a transfer vector:

The β2M coding DNA sequence is inserted into the BgIII-Kpn1 sites of the pTen12 transfer vector (Quantum). The recombinant clone is verified by enzymatic digestion and then amplified and sterilized for the purpose of cotransfection with the DNA of the linearized BacTen baculovirus.

b- Insertion of the gene encoding HLA-G5 into a transfer vector:

The sequence encoding the HLA-G5 molecule is inserted into the BgIII-Kpn1 sites of the pTen12 transfer vector. The recombinant clone is verified by enzymatic digestion and then amplified and sterilized for the purpose of cotransfection with the DNA of the linearized BacTen baculovirus (Quantum).

2. Construction of recombinant baculoviruses

The first step consists in producing, firstly, HLA-G5A recombinant baculoviruses and, secondly, β2M recombinant baculoviruses. The two transfer vectors carrying the β2M and HLA-G5A genes are placed together with linear BacTen in order to cotransfect Sf9 insect cells in culture.The cotransfection supernatants are harvested and lysis plaque cloning is carried out in

order to isolate the recombinant baculovirus clones. In order to guarantee the quality of the construct, the DNA of the viral clones is extracted and the insertion of the gene into the correct viral *locus* is verified by PCR. Six clones were obtained for each of the constructs, and each one was used to reinfect Sf9 cells. The culture supernatants and also the cells where then recovered after centrifugation. This protein is purified by immunoaffinity using PAG5-6 antibodies (see Example 4). The structure of the protein is verified by *western blot*, both with PAG5-6 antibodies (anti-HLA-G5) and BIG6 antibodies (Immunotech) (anti-β2M) specific, respectively, for the soluble HLA-G5 form and for β2M. One clone was selected from the 6 clones producing HLA-G5 and one clone was selected from the 4 clones producing β2M. One HLA-G5α clone and one β2M clone were used to coinfect Sf9 cells. It is possible to show, by western blot analysis and immunoprecipitation with a conformational antibody W6/32 (IgG2a, against HLA class I α chains associated with β2-m (Sigma)), that the supernatant from this coinfection contains the HLA-G5 protein associated with β2M; it is thus in a form close to the physiological form. These Sf9 cells therefore make it possible to obtain large amounts of soluble HLA-G5 protein.

EXAMPLE 2 : Demonstration of the regulatory role of HLA-G in the T-dependent phenomena linked to psoriasis

1. Origin of the skin biopsies

Skin biopsies exhibiting plaques typical of chronic psoriasis lesions, derived from patients who had undergone no local or general treatment during the 15 days preceding the day of the biopsy, were analyzed.

Healthy skin biopsies derived from patients who have undergone reductive breast surgery are used as a negative control.

The samples are separated into two, one portion is immediately frozen in liquid nitrogen for the RT-PCR analysis and the remaining portion is embedded in OCT (Miles Inc Diagnostics Division) for the immunohistochemical analysis.

2. Demonstration of all of the HLA-G transcripts and of the transcript specific for the soluble HLA-G5 isoform in the skin biopsies exhibiting psoriasis lesions

a) Materials and methods

The messenger RNAs are extracted from frozen biopsies of 6 specimens of lesioned psoriatic skin (Pso1-Pso-6, figures 1 and 3) and of 4 specimens of normal skin (healthy skin 1-healthy skin 4, figures 2 and 3) under the following conditions: the samples are placed together with the *RNA Now* reagent (Biogentex, Inc.) and homogenized using an *ultraturrax* homogenizer (IKA Labortechnic), according to the manufacturer's recommendations. The quality of the extracted RNA is verified by electrophoresis on a 1.5% agarose gel under denaturing conditions. 5 µg of RNA are reverse transcribed into cDNA using an oligo dT primer 12 to 18 oligonucleotides long (Gibco BRL) and the Moloney virus (M-MLV) reverse transcriptase, for 1 h at 42°C. The cDNA obtained is then amplified by PCR with, firstly, primers which recognize all of the HLA-G transcripts [primer G.257 (exon 2) and 3G.U (untranslated 3' end)], used for the PCR amplification of the HLA-G transcripts corresponding to the various known isoforms of HLA-G (figures 1 and 2) and, secondly, primers specific for the soluble HLA-G5 sequence, namely primers G.526 and G.i4b (figure 3).

More precisely, the various primers and probes used are, consequently, as follows:

- G.526 (exon 3) and G3.U (3'UT) for the G1, G4 and G5 isoforms;

- G.526 (exon 3) and G.i4b (intron 4) for the G5 isoform;

- G.-3 (partially covering exons 2 and 4) and G3.U (3'UT) for the G2 and G6 isoforms;

- G.3-4 (partially covering exons 2 and 5) and G3.U (3'UT) for the G3 isoform.

The PCR conditions are as follows: 1 minute at 90°C, 90 s at 65°C (61°C for the pair of primers G.526 and G.i4b) and 2 minutes at 72°C, for 35 cycles. The PCR products are separated by electrophoresis on a 1.5% agarose gel.

β-actin transcripts are coamplified, for 16 cycles using specific primers (Clontech), in order to standardize the amount of RNA between the various samples.

The PCR products are then transferred onto a nylon membrane (*Hybond N*$^+$, Amersham) and hybridized with a radiolabeled probe, and the intensity of the hybridization signal is quantified by densitometry.

The specific HLA-G probes are as follows:

- GR specific for exon 2, which recognizes all the HLA-G transcripts, and

- G.I4 F specific for intron 4, which only recognizes the HLA-G5 transcript.

The primers and probes above are described in P. MOREAU et al., C.R. Acad. Sci. Paris, Sciences de la vie/Life Sciences, 1995; 318; 837-42).

The cDNA from JEG-3 choriocarcinoma cells (ATCC), which have a high level of HLA-G transcription, is used as a positive control. In the assays above, the JEG-3 line is cultured in a DMEM medium (Sigma) supplemented with 10% heat-inactivated fetal calf serum, antibiotics and 2 mM L-glutamine. The cell lines do not contain microplasmas.

The specific HLA-G bands are revealed by hybridization with the GR-specific probe located in exon 2. The PCR products, coamplified during the same reaction, with the β-actin primers are detected on the same membrane using a β-actin probe.

b) Results

The results are illustrated in figures 1 to 3.

The HLA-G transcripts are detected in all the samples of lesioned skin exhibiting psoriasis lesions and in only 2 of the 4 samples of healthy skin. With the exception of one sample (sample 3), only the transcripts corresponding to the HLA-G1 and HLA-G5 isoforms are detected in the samples of lesioned skin. In the lesioned skin biopsies from 6 patients suffering from psoriasis, a higher HLA-G transcriptional level exists, in particular with regard to the G1/G5 isoform ($p<0.05$; figures 1 and 3).

In these samples, the signal for the soluble HLA-G5 isoform is absent from the 4 specimens of normal skin (figure 3), but is found in 3 of the 6 individuals exhibiting psoriasis (figure 3).

The set of results shows that the level of the HLA-G1 and -G5 transcripts is higher in the skin biopsies exhibiting psoriasis lesions than in the samples of healthy skin, and that the HLA-G5 transcript is

expressed only in the skin biopsies exhibiting psoriasis lesions.

3. Demonstration of the HLA-G protein in the skin biopsies exhibiting psoriasis lesions

a) Materials and methods

a_1) Immunohistochemistry

Cryostat sections were prepared from frozen samples and the sections were then dehydrated in acetone at -20°C for 20 min and dried in the open air.

The immunolabeling is carried out using the *Dako EnVision+System Peroxide (AEC)* kit (Dako), according to the manufacturer's instructions.

The antibodies used are as follows:

- 87G and O1G specific for HLA-G (HLA-G1 to -G5) and 16G1 specific for HLA-G5 (D. Geraghty, Fred Hutchinson Cancer Research Center, Seattle, USA),

- 4H84 specific for the isoforms of HLA-G in the denatured form (M. McMaster, San Francisco, USA) - anti-ILT2 which recognizes a KIR involved in binding to HLA-G (Navarro et al., Eur. J; Immunol., 1999, 29, 277-283),

- W6/32: anti-MHC class I antigen (Sigma)

- anti-CD3 (Sigma)

- anti-CD14 (Sigma)

- mouse IgG2a: control antibody (Sigma),

a_2) Double labeling

The double labeling is carried out with the following pairs of antibodies:

- 87G and anti-CD68

- 87G and anti-CD11c (Dako)

- anti-ILT2 and anti-CD3.

The conditions used are as follows: after incubation with normal goat serum, the sections are incubated with the first antibody (87G or anti-ILT2) for 60 min, rinsed and then incubated with a Texas red-coupled goat anti-mouse IgG antibody. The sections are then rinsed and incubated for 30 min with the second antibody coupled to fluorescein isothiocyanate (FITC): anti-CD68 or anti-CD11c antibody when the first antibody is 87G, or anti-CD3 antibody when the first antibody is anti-ILT2.

In the control sections, the first antibody (87G or anti-ILT2) is replaced with the control antibody (mouse IgG2a).

The sections are then analyzed by laser confocal microscopy.

b) Results

b_1) Detection of all the HLA-G proteins and of the soluble HLA-G5 protein in the skin biopsies

No expression of the HLA-G protein is detected in the samples of healthy skin when using the 87G antibody or the 01G antibody which recognize all the isoforms of HLA-G.

On the other hand, HLA-G expression is detected in cells of the dermal papillae in the 9 samples of skin exhibiting psoriasis lesions. The level of HLA-G expression is variable depending on the samples; it is high in some samples. In addition, in only two samples, HLA-G expression is also detected in the epidermis, in areas close to the inflammatory cell infiltrate of the dermis, either in isolated loci of the inflammatory cells or in a few keratinocytes. The localization of HLA-G in these few keratinocytes is probably linked to the diffusion of the HLA-G5 isoform from the cells of the dermis expressing said isoform.

Specific labeling of the cells of the dermal papille is also detected when using the 16G1 antibody which specifically recognizes the soluble HLA-G5 isoform.

b_2) Localization of the cells expressing HLA-G in the psoriasis lesions

Double labeling with the 87G antibody and the anti-CD3 antibody or the anti-CD14 antibody shows that HLA-G is colocalized in the cells expressing CD14; on the other hand, no colocalization of HLA-G with T lymphocytes ($CD3^+$) is observed.

b_3) The cells expressing HLA-G are $CD68^+$, $CD11c^+$ macrophages

The labeling of serial sections of the samples of skin exhibiting psoriasis lesions, with the 87G antibody and the anti-CD68 or anti-CD11c antibodies which specifically recognize macrophages, demonstrates that virtually all of the cells which express HLA-G also express CD68 and CD11c and are therefore macrophages.

b_4) The ILT2-type KIR receptor is present in the T lymphocytes infiltrating psoriasis lesions

The labeling of skin biopsies exhibiting psoriasis lesions, with the anti-ILT2 antibody, shows the presence of a dense infiltrate in the superficial dermis. On the other hand, no labeling is detected in the dermis of the samples of healthy skin. In addition, double labeling experiments with the anti-CD3 and anti-ILT2 antibodies show the presence of a few double-labeled cells in the dermal papillae.

In summary, the set of results presented above shows that:

- in healthy skin, the expression of the HLA-G RNAs is low or undetectable and the HLA-G protein is absent,

- in psoriasis lesions, there is increased expression of the RNAs and of the protein of the HLA-G1 and -G5 isoforms,

- in psoriasis lesions, the HLA-G expression is localized in macrophages, and

- in psoriasis lesions, the infiltrating T lymphocytes express a receptor for inhibiting cytotoxic functions which is recognized by HLA-G (ILT2 receptor).

These results show the presence, in psoriasis lesions, of both cells expressing HLA-G (macrophages of the dermal papillae) and infiltrating cells responsible for the psoriasis lesions ($CD3^+$ T lymphocytes), expressing a receptor for inhibiting cytotoxic functions which is recognized by HLA-G (ILT2 receptor).

Consequently, these results demonstrate the direct role of HLA-G, and in particular of the predominant membrane-bound isoform HLA-G1 and of the diffusible soluble isoform HLA-G5, in the local regulation of the T-dependent phenomena responsible for psoriasis.

EXAMPLE 3: Role of the α1 extracellular domain of HLA-G in the inhibition of immune functions, and application in the treatment of psoriasis

1. Role of the α1 extracellular domain of HLA-G in the inhibition of the cytotoxic activity of NK cells and of T cells

a) NK cells

The use of cells transfected with each of the HLA-G1, -G2, -G3 or -G4 isoforms as target cells opposite immunocompetent *natural killer* cells present in peripheral blood makes it possible to demonstrate that each of the isoforms is capable of inhibiting the cytotoxic activity of *natural killer* cells (fig. 4). These experiments were carried out on more than ten healthy voluntary donors and the significance of the inhibition exerted by each of the isoforms was validated with statistical tests (fig. 4).

b) T cells

Similar *in vitro* cytotoxicity assays carried out with the same target cells opposite MHC-restricted CD8+ T cells specific for an antigenic peptide also demonstrated that each of the HLA-G1, -G2, -G3 and -G4 isoforms significantly inhibits the cytotoxic activity of these T cells (fig. 5).

Based on the structure of the HLA-G3 isoforming consist only of the α1 extracellular domain possessing all the inhibitory properties described above, a conclusion may be drawn regarding the functionality of this domain. This domain therefore contains the functional motif of HLA-G and may therefore be used as a pharmacological agent for the purpose of immunotolerance.

2. Application in the treatment of psoriasis

The direct role of HLA-G, and in particular of the predominant membrane-bound isoform HLA-G1 and of the diffusible soluble isoform HLA-G5, in the local regulation of the T-dependent phenomena responsible for psoriasis was demonstrated in example 2.

The role of HLA-G, and in particular of the α1 domain of said isoforms, in the inhibition of T functions was demonstrated above.

Consequently, the set of results indicates that a pharmaceutical composition containing said HLA-G isoform, in particular in diffusible soluble form, such as the HLA-G5 isoform, has a protective role in the treatment of psoriasis.

EXAMPLE 4: Production of an antibody, named PAG5-6, which specifically recognizes the soluble forms of HLA-G (HLA-G5 and HLA-G6), in the form of a polyclonal serum produced in rabbits.

The immunization of rabbits with an immunogenic peptide consisting of a 21 amino acid synthetic peptide corresponding to the C-terminal portion encoded by intron 4 of the soluble HLA-G forms, the sequence of which is SKEGDGGIMSVRESRSLSEDL (SEQ ID NO:1), coupled to the KLH carrier protein, makes it possible to produce a polyclonal serum which specifically recognizes the soluble forms of HLA-G (HLA-G5 and HLA-G6) via immunoprecipitation techniques, immuno-imprinting techniques (Western blot), immunohisto-chemistry techniques and immunoenzymatic techniques of the ELISA type. The serum is purified on an affinity column (protein G-sepharose) and may be used both for detecting, titrating and purifying the soluble HLA-G forms.

As emerges from the above, the invention is in no way limited to its methods of implementation, preparation and application which have just been described more explicitly; on the contrary, it encompasses all the variants thereof which may occur to those skilled in the art, without departing from the context or scope of the present invention.